



# ON THE VARIATION OF THE HAEMOLYTIC COMPLEMENT IN EXPERIMENTAL TRYPANOSOMIASIS

BY

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Hartoch and Yakimoff\* observed a diminution in the amount of haemolytic complement in the blood of guinea-pigs and rats infected with various strains of trypanosomes shortly before the death of the animals.

With a view to ascertaining whether this diminution of the haemolytic complement bore any definite relation to the number of trypanosomes present in the peripheral blood of the infected animal, or whether it was simply a terminal event of the disease, the following series of experiments was undertaken. †

For the purpose of preparing a haemolytic system about 50 c.c. of goat's red corpuscles were injected into the peritoneal cavity of a rabbit, all traces of serum having been previously removed from the red cells by washing three or four times with 0·9 per cent. NaCl solution. These injections were repeated once or twice at intervals of seven days. Usually at the end of two or three weeks the rabbit's serum was strongly haemolytic for goat's red cells. The immune rabbit-serum was then inactivated by heating to 56°-58° C. for half an hour.

In order to standardise the haemolytic system, amounts varying

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† The animal experiments recorded in this paper were kindly performed for me by Dr. Anton Breinl.

from 0.0005 c.c.-0.005 c.c. of the immune serum were added to 1 c.c. of a 5 per cent suspension of well-washed goat's red cells. Afterwards, 1 c.c. of a 1 in 10 dilution of normal guinea-pig serum was added, as complement, to each of these mixtures of amoceptor and red cells. The contents of each of the tubes was then made up to 3 c.c. by the addition of 0.9 per cent. NaCl solution. It was found that 0.001 c.c. of the immune serum was the smallest amount necessary to haemolys 1 c.c. of a 5 per cent. suspension of goat's red cells in from 10-15 minutes at 37° C.

A haemolytic system consisting of the double amount of the immune serum 0.002 c.c. and 1 c.c. of a 5 per cent. suspension of goat's red cells was employed in the following experiments.

The serum of infected guinea-pigs was used throughout these experiments. The amount of complement present in the blood, withdrawn from one of the veins in the ear, was estimated before injection, and the guinea-pigs were then inoculated with different strains of trypanosomes, e.g., *T. gambiense*, *T. equiperdum*, *T. brucei* or *T. evansi*. Subsequently, examinations of the amount of haemolytic complement in their serum were made at intervals of two or three days during the course of the infection. An observation of the number of trypanosomes in the peripheral blood was made daily.

In Tables I and II the results obtained with the serum of guinea-pigs infected with *T. brucei* and *T. evansi*, respectively, are recorded.

Similar results were obtained with guinea-pigs 13 and 14 infected with *T. gambiense*, and with guinea-pigs 15 and 16 infected with *T. equiperdum*. The amount of haemolytic complement present in the serum of these animals was examined frequently during the period—between three and four and a half months—which they lived after infection. At no time was any diminution of the complement observed until a few hours before the animal's death, when a partial disappearance of complement was noticed in animals 13 and 15. There was no appreciable loss of complement observed in guinea-pigs 14 and 16 in the serum removed about twelve hours before their death. At no time did their blood exhibit more than one or two trypanosomes to a field, and frequently none were seen after a careful search.

TABLE I.—Guinea-pigs infected with *T. brucei*.

No. of experiment	Day	No. of parasites present in blood	0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
1	5 days before inoculation	Inoculated intraperitoneally with <i>T. brucei</i>			Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Almost complete haemolysis in 20 mins.	Trace of haemolysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation				15 "	15 "	20 "	3 "	No haemolysis in 3 hours
2nd	"	5 parasites to a field	"	"	"	"	"	"	"
3rd	"	"	"	"	"	"	"	"	"
4th	"	"	"	"	"	"	"	"	"
5th	"	"	"	"	"	"	"	"	"
6th	"	"	"	"	"	"	"	"	"
7th	"	50 Parasites	"	"	"	"	"	"	"
8th	"	numerous	"	"	"	"	"	"	"
9th	"	"	"	"	"	"	"	"	"
10th	"	"	"	"	"	"	"	"	"
11th	"	40 to field			Complete haemolysis in 10 mins.	Complete haemolysis in 10 mins.	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours
12th	"	Numerous*			No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours
13th	"	Found dead							

\* Animal very ill at the time the serum was removed.

TABLE I—*continued.*

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amoebocyte and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						No. haemolysis in 3 hours
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	
2	2 days before inoculation								
		Inoculated intraperitoneally with <i>T. brucei</i>							
	1st day after inoculation								
2nd	"	5 to a field	"	15 "	"	"	20 "	"	3 "
3rd	"	2 "	"	"	"	"	"	"	3 "
4th	"	5 "	"	"	"	"	"	"	3 "
5th	"	10 "	"	15 "	"	"	20 "	"	3 "
6th	"	15 "	"	"	"	"	"	"	3 "
7th	"	5 "	"	"	"	"	"	"	3 "
8th	"	5 "	"	15 "	"	"	20 "	"	3 "
9th	"	1 to 5 fields	"	"	"	"	"	"	3 "
10th	"	1 to 20 fields	"	"	"	"	"	"	3 "
11th	"	1 to 5 "	"	"	"	"	"	"	3 "
12th	"	1 to a field	"	15 "	"	"	20 "	"	3 "
13th	"	5 "	"	"	"	"	"	"	3 "
14th	"	10 "	"	15 "	"	"	20 "	"	3 "
15th	"	15 "	"	"	"	"	"	"	3 "
16th	"	40 "	"	Complete haemolysis in 10 mins.	"	"	20 "	"	3 "
17th	"	Numerous "	"	15 "	"	"	20 "	"	3 "
18th	"	"	No haemolysis in 3 hours				No haemolysis in 3 hours		No haemolysis in 3 hours
19th	"	Innumerable*	Slight haemolysis in 3 hours				No haemolysis in 3 hours		No haemolysis in 3 hours
20th	"	Found dead							

\* Animal very ill at the time the serum was removed.

TABLE I—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amoceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
3	1st day after inoculation	Inoculated intraperitoneally with <i>T. brucei</i>		Complete haemolysis in 10 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours
2nd									
3rd									
4th			5 to a field	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
5th			30 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
6th			20 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
7th			1 to 5 fields						
8th			1 to 20 fields						
9th			1 to a field						
10th			5 "						
11th			10 "						
12th			15 "						
13th			40 "						
14th			Innumerable	Complete haemolysis in 20 mins.	" 60 "	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours
15th									
16th									
17th									
18th									
19th				No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	" 3 "	" 3 "

\* Animal dying when the serum was withdrawn.

TABLE I.—*continued.*

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
4	Inoculated intraperitoneally with <i>T. brucei</i>	1st day after inoculation	Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 30 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours	0/1
			" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "	" 3 "
			1 to 2 fields	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			10 to a field	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			20 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			30 "						
			20 "						
			20 "						
			Found dead						

TABLE I. *continued.*

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.													
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.							
5	1st day after inoculation	Inoculated intraperitoneally with <i>T. brucei</i>	Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 30 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours	571							
	2nd	4 to film														
	3rd	10 "		15 "												
	4th	40 "														
	5th	"														
	6th	"														
	7th	Numerous														
	8th	"		15 "												
	9th	"														
	10th	"														
	11th	"														
	12th	"														
	13th	"														
	14th	1 to a field														
	15th	2 "														
	16th	5 "														
	17th	10 "		15 "												
	18th	15 "														
	19th	"														
	20th	40 "		15 "												
	21st	Found dead														
6	1st day after inoculation	Inoculated intraperitoneally with <i>T. brucei</i>	Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 30 mins.	Marked haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	571							
7	2nd	1 to a field														
7	3rd	10 "														
7	4th	50 "														
7	5th	50 "														
7	6th	Numerous*														

\* Animal dying when the serum was withdrawn.

TABLE I *continued.*

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
7		Inoculated intraperitoneally with <i>T. brucei</i>		Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours
	1st day after inoculation								
2nd	"								
3rd	"								
4th	"	10 to a field		" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
5th	"								
6th	"	5 "							
7th	"	1 to 5 fields		" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
8th	"	1 to 10 "							
9th	"	1 to 5 "							
10th	"								
11th	"	1 to a field							
12th	"	10 "							
13th	"	10 "							
14th	"	10 "							
15th	"	10 "							
16th	"	15 "							
17th	"	40 "							
18th	"	Numerous	Complete haemolysis in 10 mins.	" 15 "	" 15 "	" 20 "	" 3 "	No haemolysis in 3 hours	" 3 "
19th	"	15 to a field							
20th	"	50 "							
21st	"	Numerous		" 20 "	" 20 "	" 30 "	" 3 "	Slight haemolysis in 3 hours	" 3 "
22nd	"	" * "	" 10 "	" 15 "	" 15 "	" 20 "	" 3 "	" 3 "	" 3 "
23rd	"	" * "	" 10 "	" 60 "	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	" 3 "

\* Animal dying when the serum was withdrawn.

TABLE I—*continued.*

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.			
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.
8		Inoculated intraperitoneally with <i>T. brucei</i>			Complete haemolysis in 15 mins.	Complete haemolysis in 15 mins.
	1st day after inoculation				Complete haemolysis in 30 mins.	Complete haemolysis in 30 mins.
2nd	1 to 20 fields				30	30
"	1 to 10 "				30	30
3rd	5 to field				30	30
4th	2 "				30	30
5th	15 "				30	30
6th	15 "				30	30
7th	5 "				30	30
8th	1 "				30	30
9th	5 "				30	30
10th	15 "				30	30
11th	Numerous				30	30
		Complete haemolysis in 10 mins.			30	30
12th	"				30	30
13th	"				30	30
14th	"	Found dead			30	30

TABLE I (continued)

Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amoebocyte and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.

No. of experiment	Day	No. of parasites present in blood	C. 5 c.c.				0.1 c.c.				0.075 c.c.				0.05 c.c.				0.025 c.c.				0.01 c.c.				0.005 c.c.			
			Inoculated intraperitoneally with <i>T. brucei</i>				Complete haemolysis in 15 mins.				Complete haemolysis in 30 mins.				Almost complete haemolysis in 3 hours				Trace of haemolysis in 3 hours				No haemolysis in 3 hours							
1st day after inoculation																														
1	2nd	"	1 to 40 fields																											
	3rd	"	1 to 2 film																											
	4th	"	1 to 5 fields																											
	5th	"	1 to 15 fields																											
	6th	"	1 to 30 fields																											
	7th	"	1 to 4 film																											
	8th	"	1 to 5 fields																											
	9th	"	5-10 to 1 field																											
	10th	"	1 to 2 fields																											
	11th	"	1 to 1 film																											
	12th	"	1 to 2 film																											
	13th	"	1 to 1 film																											
	14th	"	1 to 2 film																											
	15th	"	1 to film																											
	16th	"	1 to film																											
	17th	"	Negative																											
	18th	"																												
	19th	"																												
	20th	"																												
	21st	"																												
	22nd	"																												
	23rd	"																												
	24th	"																												
	25th	"																												
	26th	"																												
	27th	"																												
	28th	"																												
	29th	"																												
	30th	"																												
	31st	"																												
	32nd	"																												
	33rd	"																												
	34th	"																												
	35th	"																												
	36th	"																												
	37th	"																												

\* Animal moribund at the time the serum was withdrawn  
† No haemolysis in 3 hours

TABLE II.—Guinea-pigs infected with *T. evansi*.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
10	Inoculated intraperitoneally with <i>T. evansi</i>	1st day after inoculation 2nd .. Negative 3rd .. 1 to field 4th .. 5 .. 5th .. 10 .. 6th .. Negative 7th .. 2 to film 8th .. 1 to 2 fields 9th .. 20 to field Found dead	Complete haemo-	Complete haemo-	Complete haemo-	Complete haemo-	Almost complete	Slight haemo-	No haemolysis
			lysis in 15 mins.	lysis in 15 mins.	lysis in 15 mins.	lysis in 30 mins.	haemolysis in 3 hours	lysis in 3 hours	in 3 hours
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
11	Inoculated intraperitoneally with <i>T. evansi</i>	1st day after inoculation 2nd .. 3rd .. 5 to field 4th .. 15 .. 5th .. 15 .. 6th .. 10 .. 7th .. Found dead	Complete haemo-	Complete haemo-	Complete haemo-	Complete haemo-	Marked haemo-	No haemolysis in	No haemolysis
			lysis in 10 mins.	lysis in 15 mins.	lysis in 20 mins.	lysis in 30 mins.	lysis in 3 hours	3 hours	in 3 hours
			" 15 "	" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "
			" 15 "	" 15 "	" 15 "	" 30 "	" 3 "	" 3 "	" 3 "

TABLE II—continued.

No. of experiment	Day	No. of parasites present in blood	Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amoebocyte and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution.						
			0.5 c.c.	0.1 c.c.	0.075 c.c.	0.05 c.c.	0.025 c.c.	0.01 c.c.	0.005 c.c.
12		Inoculated intraperitoneally with <i>T. evansi</i>	Complete haemolysis in 10 mins.	Complete haemolysis in 15 mins.	Complete haemolysis in 20 mins.	Complete haemolysis in 30 mins.	Almost complete haemolysis in 3 hours	Slight haemolysis in 3 hours	No haemolysis in 3 hours
1st day after inoculation	Negative								
2nd	"								
3rd	"	2 to film		" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
4th	"								
5th	"	1 to field							
6th	"								
7th	"	15 "		" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
8th	"	15 "		" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
9th	"	15 "		" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
10th	"	20 "		" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
11th	"	30 "		" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
12th	"	2 "		" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
13th	"	1 to 5 fields							
14th	"	1 to 10 fields							
15th	"	5 to field							
16th	"	1 "							
17th	"	3 "							
18th	"	4 to film							
19th	"	5 "							
20th	"	Negative							
21st	"			" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
22nd	"			" 15 "	" 20 "	" 30 "	" 3 "	" 3 "	" 3 "
23rd	"	2 to film							
24th	"	2 "							
25th	"	Negative							
26th	"	1 to 5 fields							
27th	"								
28th	"	10 to field		" 10 "	" 15 "	" 20 "	" 30 "	" 3 "	" 3 "
29th	"	60 "							
30th	"	Numerous *	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours	No haemolysis in 3 hours

\* Animal dying at the time the serum was withdrawn.

In none of the foregoing experiments was any decrease in the amount of haemolytic complement observed until shortly before the death of the animal, with the single exception of Experiment 1. The serum of this guinea-pig, which was infected with *T. brucei*, showed a slight decrease in complement on the 8th and 11th day of the disease. The blood at the time contained numerous trypanosomes.

In Experiments 2, 5, 7 and 8 no appreciable decrease of the haemolytic complement occurred during the course of the disease, even when the parasites were swarming in the peripheral blood (1 to every 2 or 3 red cells). Many estimations of the complement were made in the different experiments, when 20-50 parasites were present to the microscopic field (Zeiss objective DD; eyepiece No. 4). In no case was any diminution noticed. It is evident, therefore, that the presence of numerous trypanosomes in the peripheral blood in an early stage of the disease does not necessarily result in a diminution of the haemolytic complement.

On the other hand in a late stage of the infection shortly before the death of the animal, a considerable decrease in the amount of complement was observed in most of the experiments. On five occasions, Experiments 1, 2, 3, 9 and 12, the blood of animals infected with *T. brucei* and *T. evansi*, respectively, contained practically no haemolytic complement a few hours before death. In other cases, e.g., Experiments 6, 7, 13 and 15, only a partial diminution could be observed in the serum removed just before the death of the animal, whilst in still other cases, 5, 10, 11, 14 and 16, no decrease of the haemolytic complement was found in the serum withdrawn about twelve hours before death.

From these observations one must conclude, therefore, that the diminution of the complement in the serum of animals infected with trypanosomiasis is simply a terminal event, and does not occur during the earlier stages of the disease, even when the peripheral blood is swarming with parasites.

The following experiments were performed with a view to ascertaining whether the serum of infected guinea-pigs withdrawn a few hours before their death contained any body which had the property of inhibiting to any extent the activating power of the complement of normal serum.

EXPERIMENT.—The serum of guinea-pig 3 was obtained shortly before the death of the animal. It had not the slightest activating action even when added undiluted to the standardised haemolytic system of goat's red cells and amboceptor. A portion of this serum was added in varying proportions to the serum of a normal guinea-pig. The resulting mixtures were then kept at 37° C. for 20 minutes before being added to the haemolytic system. A second portion of the serum of guinea-pig 3 was first heated to 56°-58° C. for half an hour in order to destroy any complement it might contain, and then, as above, mixed in different proportions with normal guinea-pig serum.

The following Table gives the result of this experiment :

A					
	5% suspension of goat's red cells	Amboceptor	COMPLEMENT		Haemolysis
			Normal guinea-pig serum	0.9% NaCl solution	
1	1.0 c.c.	0.002 c.c.	0.1 c.c.	0.1 c.c.	Complete haemolysis in 15 mins.
2	"	"	0.05 c.c.	0.15 c.c.	Complete haemolysis in 30 mins.
3	"	"	0.025 c.c.	0.175 c.c.	Partial haemolysis in 3 hours
4	"	"	0.01 c.c.	0.19 c.c.	Trace of haemolysis in 3 hours

  

B					
	5% suspension of goat's red cells	Amboceptor	COMPLEMENT		Haemolysis
			Normal guinea-pig serum	Serum guinea-pig 3	
1	1.0 c.c.	0.002 c.c.	0.1 c.c.	0.1 c.c.	Complete haemolysis in 15 mins.
2	"	"	0.05 c.c.	0.15 c.c.	Complete haemolysis in 30 mins.
3	"	"	0.025 c.c.	0.175 c.c.	Partially complete haemolysis in 3 hours
4	"	"	0.01 c.c.	0.19 c.c.	Slight haemolysis in 3 hours

  

C					
	5% suspension of goat's red cells	Amboceptor	COMPLEMENT		Haemolysis
			Normal guinea-pig serum	Serum of guinea-pig 3 (heated 56-58° C. for 20 mins.)	
1	1.0 c.c.	0.002 c.c.	0.1 c.c.	0.1 c.c.	Complete haemolysis in 15 mins.
2	"	"	0.05 c.c.	0.15 c.c.	Complete haemolysis in 30 mins.
3	"	"	0.025 c.c.	0.175 c.c.	Partial haemolysis in 3 hours
4	"	"	0.01 c.c.	0.19 c.c.	Trace of haemolysis in 3 hours